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Steroidal glycosides from the underground parts of *Dracaena thalioides* and their cytotoxic activity

Liying Tang [a,b], Zhuju Wang [a], Hongwei Wu [a], Akihito Yokosuka [b,*], Yoshihiro Mimaki [b]

[a] Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, No.16 Nanxiaojie, Dongzhimennei, Beijing 100700, China
[b] Department of Medicinal Pharmacognosy, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan

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ABSTRACT

Six spirostanol glycosides (**1**–**6**) and 12 known compounds (**7**–**18**) were isolated from the underground parts of *Dracaena thalioides* (Agavaceae). Their structures were determined by spectroscopic analysis, including 2D NMR spectroscopic data, and chemical transformations. The isolated compounds were evaluated for cytotoxic activity against HL-60 human leukemia cells. Compounds **1**, **3**–**6**, and **8**–**18** showed cytotoxicity against HL-60 cells, of which **10**, a bisdesmosidic spirostanol derivative, showed potent cytotoxicity against HL-60 cells with an IC_{50} value of 0.38 μM and induced apoptosis in HL-60 cells.

1. Introduction

Plants of the genus *Dracaena* (Agavaceae) comprise about 50 species known to be rich sources of steroidal saponins. Previously, the chemical components of several *Dracaena* plants were examined, and a variety of steroidal glycosides were isolated, some of which showed cytotoxic activities against cultured tumor cells (Mimaki et al., 1999, 2001; Yokosuka et al., 2000; Yokosuka and Mimaki, 2009). *Dracaena thalioides* hort. Makoy ex E. Morr. is a perennial plant mainly found in tropical West Africa (Tsukamoto, 1988). Previously, four steroidal glycosides, three glyceroglycolipid-related compounds, and two flavonol glycosides from the leaves of *D. thalioides* were reported (Yokosuka et al., 2013). Phytochemical studies of the underground parts of this plant herein resulted in the isolation of 6 new spirostanol glycosides (**1**–**6**) together with 12 known compounds (**7**–**18**). The structures of **1**–**6** were determined by spectroscopic analysis, including 2D NMR spectroscopic data, and chemical transformations. The cytotoxic activity of the isolated compounds against HL-60 human leukemia cells was also evaluated.

2. Results and discussion

2.1. Structural elucidation

The fresh underground parts of *D. thalioides* (8.7 kg) were extracted with hot MeOH. After removing the solvent, the MeOH extract was subjected to column chromatography (CC) using porous-polymer polystyrene resin (Diaion HP-20), silica gel, and octadecylsilanized (ODS) silica gel, respectively, to yield **1**–**18**. Compounds **7**–**18** were identified as (25*R*)-17α-hydroxyspirost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranoside (**7**) (Tapondjou et al., 2008), (25*R*)-17α-hydroxyspirost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-*O*-[α-L-rhamnopyranosyl-(1→3)]-β-D-glucopyranoside (**8**) (Liu et al., 2009), (25*R*)-3β-hydroxyspirost-5-en-1β-yl *O*-α-L-rhamnopyranosyl-(1→2)-*β*-D-xylopyranoside (**9**) (Wang et al., 2011), (23*S*,24*S*)-1β-[(*O*-(3-*O*-acetyl-β-D-xylopyranosyl-(1→3)-*O*-[2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-]-α-L-arabinopyranosyl)oxy]-3β,23-dihydroxyspirosta-5,25(27)-dien-24-yl β-D-fucopyranoside (**10**) (Tran et al., 2001); (23*S*,24*S*)-3β,23-dihydroxy-1β-[*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranosyl)oxy]spirosta-5,25(27)-dien-24-yl β-D-fucopyranoside (**11**) (Mimaki et al., 1996a); (23*S*,24*S*)-1β-[*O*-(2,3-di-*O*-acetyl-α-L-rhamnopyranosyl)-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranosyl)oxy]-3β,23-dihydroxyspirosta-5,25(27)-dien-24-yl β-D-fuco pyranoside (**12**) (Mimaki et al., 1996a); (23*S*,24*S*)-3β,23-dihydroxy-1β-[*O*-

* Corresponding author. Tel.: +81 42 676 4577; fax: +81 42 676 4579.
E-mail address: yokosuka@toyaku.ac.jp (A. Yokosuka).

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(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranosyl)oxy]spirosta-5,25(27)-dien-24-yl β-D-glucopyranoside (**13**) (Mimaki et al., 1996a); (23*S*,24*S*)-3β,23,24-trihydroxyspirosta-5,25(27)-dien-1β-yl *O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranoside (**14**) (Mimaki et al., 1996a); (23*S*,24*S*,25*S*)-3β,23-dihydroxy-1β-[*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranosyl)oxy]spirost-5-en-24-yl β-D-fucopyranoside (**15**) (As ano et al., 1993); 3β-hydroxyspirosta-5,25(27)-dien-1β-yl α-L-arabinopyranoside (**16**) (Monti et al., 2004); 3β-hydroxyspirosta-5,25(27)-dien-1β-yl *O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (**17**) (Mimaki et al., 1996b); and 3β-hydroxyspirosta-5,25(27)-dien-1β-yl *O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranoside (**18**) (Mimaki et al., 1996a), respectively (Fig. 1).

Compound **1** was obtained as an amorphous solid with a molecular formula of $C_{47}H_{74}O_{18}$ as determined from its HRESI-TOFMS (*m*/*z*: 949.4741 $[M + Na]^+$) and ^{13}C NMR spectroscopic data. Its IR spectrum showed a broad absorption band due to hydroxyl groups at 3416 cm^{-1}, as well as a strong absorption due to a carbonyl group at 1747 cm^{-1}. The ^1H NMR spectrum of **1** showed signals for two tertiary methyl groups at δ_H 1.06 (3H, *s*) and 0.96 (3H, *s*), two secondary methyl groups at δ_H 1.23 (3H, *d*, *J* = 7.2 Hz) and 0.69 (3H, *d*, *J* = 5.7 Hz), and an olefinic proton at δ_H 5.31 (1H, *br d*, *J* = 4.7 Hz), as well as resonanced for three anomeric protons at δ_H 5.79 (1H, *br s*), 5.66 (1H, *br s*), and 4.90 (1H, *d*, *J* = 7.7 Hz), and two methyl groups of the deoxyhexopyranosyl moieties at δ_H 1.73 (3H, *d*, *J* = 6.2 Hz) and 1.64 (3H, *d*, *J* = 5.2 Hz). Comparison of the ^{13}C NMR spectrum of **1** with that of **8** indicated that the structure of the aglycone moiety of **1** was identical to that of **8**. However, differences were observed in the glucosyl moiety (Glc) attached to C-3 of the aglycone. The presence of an acetyl group in **1** was confirmed by analysis of the IR (1747 cm^{-1}), ^1H NMR [δ_H 2.18 (3H, *s*)], and ^{13}C NMR [δ_C 170.4 (C=O) and 21.4 (CH_3)] spectra. Alkaline treatment of **1** with 3% NaOMe in MeOH furnished **8**, indicating that **1** was a monoacetate of **8**. The H-4 proton of Glc was shifted to a lower field at δ_H 5.41, and a correlation peak was observed from H-4 of Glc to the acetyl carbonyl carbon at δ_C 170.4 in the HMBC spectrum. The structure of **1** was thus assigned as (25*R*)-17α-hydroxyspirost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-*O*-[α-L-rhamnopyranosyl-(1→3)]-4-*O*-acetyl-β-D-glucopyranoside.

Compound **2** was isolated as an amorphous solid with a molecular formula of $C_{49}H_{76}O_{19}$ determined from its HRESI-TOFMS (*m*/*z* 991.4838 $[M + Na]^+$) and ^{13}C NMR spectroscopic data. The ^1H and ^{13}C NMR spectra of **2** were very similar to those of **1**. The IR (1747 cm^{-1}), ^1H NMR [δ 2.19 (3H, *s*) and 2.05 (3H, *s*)], and ^{13}C NMR [δ 170.2 and 170.6 (C=O); δ 21.3 and 20.7 (Me)] spectra showed the presence of one more acetyl moiety in **2**. Alkaline treatment of **2** with 3% NaOMe in MeOH furnished **8**. In the HMBC spectrum of **2**, correlation peaks were observed between H-4 of Glc at δ_H 5.38 and the acetyl carbonyl carbon at δ_C 170.6 and between H-6 of Glc at δ_H 4.56 and 4.35 and the acetyl carbonyl carbon at δ_C 170.2. Thus, structure **2** was established as (25*R*)-17α-hydroxyspirost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-*O*-[α-L-rhamnopyranosyl-(1→3)]-4,6-*O*-diacetyl-β-D-glucopyranoside.

Compound **3** had a molecular formula of $C_{32}H_{50}O_8$ determined from its HRESI-TOFMS (*m*/*z* 585.3400 $[M + Na]^+$) and ^{13}C NMR spectroscopic data. The ^1H NMR spectrum of **3** contained four steroidal methyl proton signals at δ_H 1.30 (3H, *s*), 1.13 (3H, *d*, *J* = 6.9 Hz), 0.86 (3H, *s*), and 0.69 (3H, *d*, *J* = 5.2 Hz), an anomeric proton resonance at δ_H 4.85 (1H, *d*, *J* = 7.6 Hz), and an olefinic proton signal at δ_H 5.59 (1H, *br d*, *J* = 5.4 Hz). When **3** was subjected to acid hydrolysis with 1 M HCl in dioxane–H_2O (1:1), it was hydrolyzed to yield D-xylose (Xyl) and a sapogenin identified as (25*R*)-spirost-5-ene-1β,3β-diol (**3a**, ruscogenin) (Agrawal et al., 1985). Identification of Xyl was performed on an amino-propyl-bonded silica gel column and the compound was detected with an optical rotation (OR) detector. In the HMBC spectrum, a correlation peak between the resonances of H-1 of Xyl at δ_H 4.85 and C-1 of the aglycone at δ_C 83.2 implied that one Xyl unit was attached at C-1 of the aglycone. Thus, **3** was identified as (25*R*)-3β-hydroxyspirost-5-en-1β-yl β-D-xylopyranoside.

Compound **4** was shown to have a molecular formula $C_{43}H_{68}O_{16}$ based on the HRESI-TOFMS (*m*/*z* 863.4412 $[M + Na]^+$) and ^{13}C NMR data. Its ^1H NMR spectrum showed signals for three anomeric protons at δ_H 6.44 (1H, *br s*), 4.94 (1H, *d*, *J* = 7.6 Hz), 4.70 (1H, *d*, *J* = 7.6 Hz), as well as resonances for four steroidal methyl groups [δ_H 1.42 (3H, *s*), 1.15 (3H, *d*, *J* = 6.9 Hz), 0.92 (3H, *s*), and 0.68 (3H, *d*, *J* = 5.2 Hz)] and an olefinic proton [δ_H 5.58 (1H, *br d*, *J* = 5.5 Hz)]. Acid hydrolysis of **4** with 1 M HCl resulted in the production of ruscogenin, D-xylose, and L-rhamnose. Analysis of the ^1H-^1H COSY, HMQC, and HMBC spectra of **4** indicated that the sugar moiety of **4** was composed of a terminal α-L-rhamnopyranosyl unit (Rha) [δ_H 6.44 (*br s*); δ_H 101.7, 72.4, 72.4, 74.1, 69.6, and 19.3], a C-2 and C-3 disubstituted β-D-xylopyranosyl unit (Xyl) [δ_H 4.70 (*d*, *J* = 7.6 Hz); δ_C 100.6, 75.9, 88.5, 69.5, and 66.5], and a terminal β-D-xylopyranosyl unit (Xyl′) [δ_H 4.94 (*d*, *J* = 7.6 Hz); δ_C 105.2, 74.7, 78.4, 70.6, and 67.2] (Agrawal et al., 1985). In the HMBC spectrum of **4**, long-range correlations were observed between: H-1 of Rha at δ_H 6.44 and C-2 of Xyl at δ_C 75.9; H-1 of Xyl′ at δ_H 4.94 and C-3 of Xyl at δ_C 88.5; and H-1 of Xyl at δ_H 4.70 and C-1 of the aglycone at δ_C 84.0. The structure of **4** was thus assigned as (25*R*)-3β-hydroxyspirost-5-en-1β-yl *O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-β-D-xylopyranoside.

Compound **5** had a molecular formula of $C_{39}H_{62}O_{13}$ determined from its HRESI-TOFMS (*m*/*z* 761.4116 $[M + Na]^+$) and ^{13}C NMR spectroscopic data. The ^1H and ^{13}C NMR properties of the aglycone moiety of **5** were in good agreement with those of **3** and **4**. Acid hydrolysis of **5** with 1 M HCl yielded ruscogenin, D-glucose, and L-rhamnose. Analysis of the ^1H–^1H COSY, HMQC, and HMBC spectra of **5** indicated that the sugar moiety of **5** was composed of a terminal α-L-rhamnopyranosyl unit (Rha) and a C-2 substituted β-D-glucopyranosyl unit (Glc). The HMBC spectrum showed correlation peaks between H-1 of Rha at δ_H 6.50 and C-2 of Glc at δ_C 76.7; and between H-1 of Glc at δ_H 4.89 and C-1 of the aglycone at δ_C 83.7. The structure of **5** was shown to be (25*R*)-3β-hydroxyspirost-5-en-1β-yl *O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside.

Compound **6** had a molecular formula of $C_{57}H_{84}O_{27}$, as determined from its HRESI-TOFMS (*m*/*z* 1223.5079 $[M + Na]^+$) and ^{13}C NMR spectroscopic data. The ^1H NMR spectrum of **6** showed signals for two tertiary methyl groups at δ_H 1.35 and 0.99 (each 3H, *s*), a secondary methyl group at δ_H 1.08 (3H, *d*, *J* = 7.0 Hz), an exomethylene group at δ_H 5.22 and 5.03 (each 1H, *br s*), an olefinic proton at δ_H 5.63 (1H, *br d*, *J* = 4.4 Hz), four anomeric protons at δ_H 6.40 (1H, *br s*), 5.38 (1H, *d*, *J* = 7.9 Hz), 4.83 (1H, *d*, *J* = 7.8 Hz), and 4.63 (1H, *d*, *J* = 7.7 Hz). The presence of four acetyl groups in **6** was confirmed by analysis of the IR (1747 cm^{-1}), ^1H NMR [δ_H 2.12 (3H, *s*), 2.04 (3H, *s*), 1.99 (3H, *s*), and 1.92 (3H, *s*)], and ^{13}C NMR [δ_C 170.5, 170.4 (x 2), and 170.3; 21.0, 20.8, 20.7, and 20.6] spectra. The data indicated that **6** was a spirostanol glycoside with four sugars and four acetyl groups. The ^1H and ^{13}C NMR spectroscopic data of **6** were closely related to those of the known compound **13**; however, differences were observed in the signals because of the β-D-xylopyranosyl (Xyl) unit between the two compounds. The ^1H-^1H COSY spectrum allowed the resonance observed at a lower field at δ_H 5.58 (dd, *J* = 9.2, 9.2 Hz) to be assigned as H-3 of Xyl, which suggested it was acetylated. This was confirmed by an HMBC correlation between H-3 in Xyl and the acetyl carbonyl carbon at δ_C 170.4. Thus, the structure of **6** was assigned as (23*S*,24*S*)-3β,23-dihydroxy-1β-[*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1→2)-*O*-

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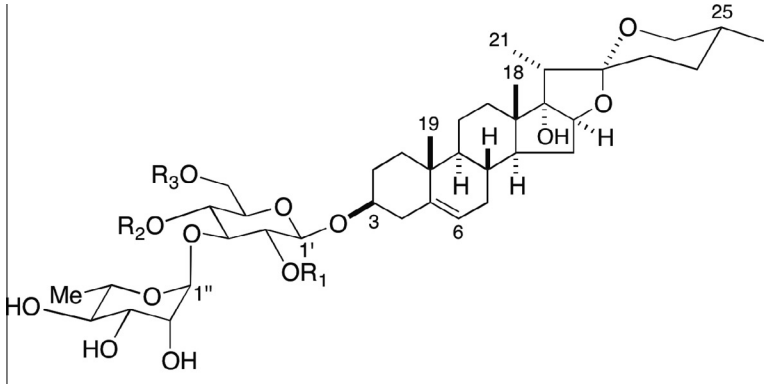
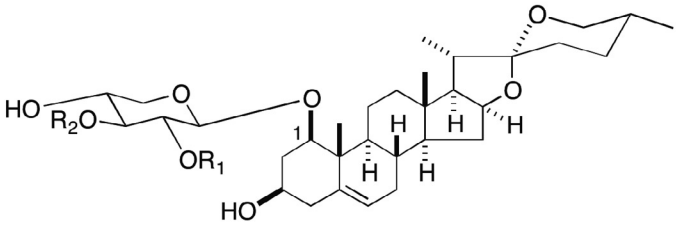


	R_1	R_2	R_3
1	Rha	Ac	H
2	Rha	Ac	Ac
7	H	H	H
8	Rha	H	H



	R_1	R_2
3	H	H
4	Rha	Xyl
9	Rha	H

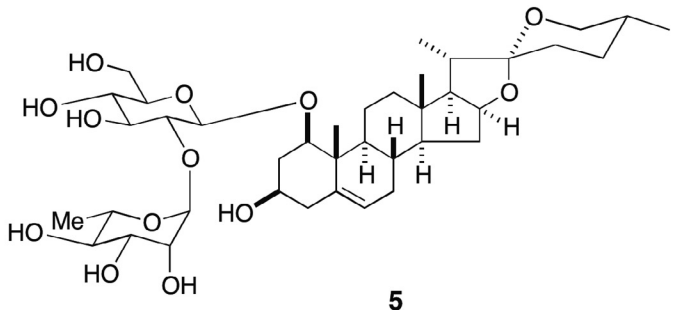


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Fig. 1. Structure of compounds **1**–**18**.

[3-*O*-acetyl-β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranosyl)ox y]spirosta-5,25(27)-dien-24-yl β-D-glucopyranoside.

2.2. Cytotoxic activity

The isolated compounds (**1**–**18**) were evaluated for cytotoxic activity against HL-60 cells (Table 3). Compounds **1**, **3**–**6**, and **8**–**18** were cytotoxic to HL-60 cells with IC_{50} values ranging from 0.38 to 17.3 μM, whereas etoposide, which was used as a positive control, gave an IC_{50} value of 0.38 μM. Compounds **10**, **11**, and **15** showed potent cytotoxic activity against HL-60 cells with IC_{50} values of 0.38, 0.47, and 0.74 μM, respectively. Compound **12** was a derivative of **11** without the acetyl group at C-4 of the α-L-rhamnopyranosyl moiety and the cytotoxicity of **12** (IC_{50}: 6.00 μM) was less potent than that of **11**. The cytotoxic activity of the 24-deglycosyl derivative **14** (IC_{50}: 4.45 μM) of **11**, and the 24-*O*-β-D-glucopyranosyl analogues **6** (IC_{50}: 1.66 μM) and **13** (IC_{50}: 2.73 μM) of **10** and **11**, respectively, were weaker than that of the parent compounds. These data suggest that the triacetylated α-L-rhamnopyranosyl moiety and the β-D-fucopyranosyl group attached to C-24

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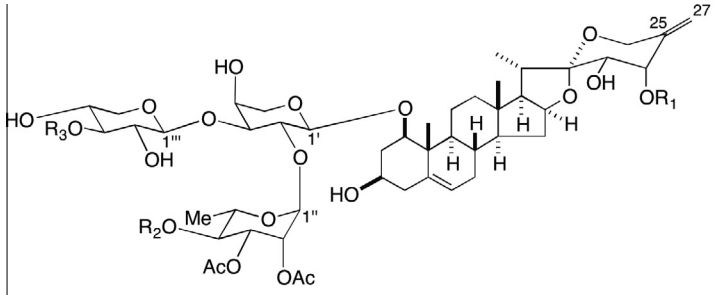
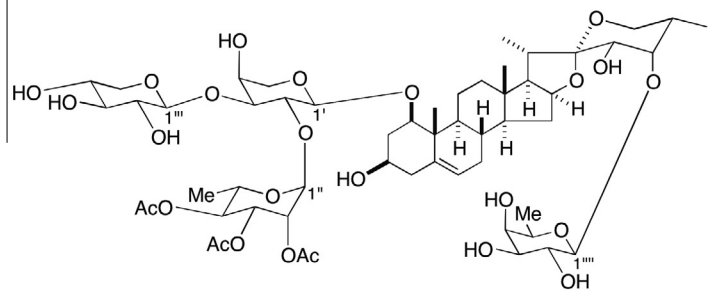


	R_1	R_2	R_3
6	Glc	Ac	Ac
10	Fuc	Ac	Ac
11	Fuc	Ac	H
12	Fuc	H	H
13	Glc	Ac	H
14	H	Ac	H



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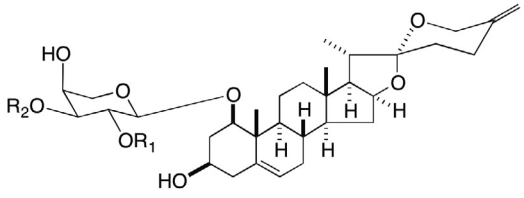

	R_1	R_2
16	H	H
17	Rha	H
18	Rha	Xyl

Fuc: β-D-fucopyranosyl
Glc: β-D-glucopyranosyl
Rha: α-L-rhamnopyranosyl
Xyl: β-D-xylopyranosyl

Fig. 1 (*continued*)

of the aglycone play important roles in the potent cytotoxic activity of **10** and **11**.

2.3. Apoptosis induction activity

Compound **10** was the most cytotoxic to HL-60 cells with an IC_{50} value of 0.38 μM. HL-60 cells treated with **10** (10 μM) for 48 h were observed by fluorescence microscopy after being stained with 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI). The cells exposed to **10** and etoposide (15 μM) displayed nuclear chromatin condensation and apoptotic bodies (Fig. 2). These morphological changes suggested that HL-60 cell death caused by **10** was partially mediated by the induction of apoptosis. In agarose gel electrophoresis of the DNA fraction of the **10**-treated HL-60 cells, a typical ladder pattern of the inter-nucleosomal fragmentation of DNA was observed (Fig. 3). Caspase-3 plays a crucial role in apoptotic signaling pathways and is the enzyme that executes apoptosis. Caspase-3 was activated when HL-60 cells were treated with **10** at a sample concentration of 10 μM for 18 h (Fig. 4).

2.4. Conclusion

The present study of the underground parts of *D. thalioides* resulted in the isolation of 18 steroidal glycosides, including 6 new spirostanol glycosides (**1**–**6**). Compounds **1**, **2**, and **6** are new spirostanol glycosides with one or more acetyl groups at their

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sugar moieties. In particular, **6** has a unique structure with four acetyl groups at its sugar moiety.

Compounds **1**, **3**–**6**, and **8**–**18** showed cytotoxic activity against HL-60 cells. Spirostanol glycoside **10** was the most cytotoxic to HL-60 cells with an IC_{50} value of 0.38 μM, and it caused apoptotic cell death in HL-60 cells via caspase-3 activation.

3. Experimental

3.1. General

Optical rotations were measured using a JASCO DIP-360 (Tokyo, Japan) automatic digital polarimeter. IR spectra were recorded on a JASCO FT-IR 620 spectrophotometer, whereas NMR spectra were acquired on a Bruker DRX-500 (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR, Karlsruhe, Germany) spectrometer using standard Bruker pulse programs. Chemical shifts are given as δ values with reference to tetramethylsilane (TMS) as an internal standard. HRESI-TOFMS was recorded on a Waters-Micromass LCT mass spectrometer (Manchester, UK). Diaion HP-20 (Mitsubishi-Chemical, Tokyo, Japan), silica gel (Fuji-Silysia Chemical, Aichi, Japan), and ODS silica gel (Nacalai Tesque, Kyoto, Japan) were used for column chromatography (CC). TLC was carried out on precoated silica gel 60 F_{254} (0.25 mm thick, Merck, Darmstadt, Germany) and RP_{18} F_{254S} plates (0.25 mm thick, Merck), and spots were visualized by spraying the plates with 10% H_2SO_4 aqueous solution, followed by heating. HPLC was performed by using a system composed of a DP-8020 (Tosoh, Tokyo, Japan) pump, a Shodex OR-2 (Showa-Denko, Tokyo, Japan) detector, and a Rheodyne injection port. HL-60 cells were obtained from the Human Science Research Resources Bank (JCRB 0085, Osaka, Japan). The following reagents were obtained from the indicated companies: RPMI 1640 medium and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) (Sigma–Aldrich, St. Louis, MO, USA); fetal bovine serum (FBS) (Nichirei Biosciences, Tokyo, Japan); penicillin G sodium salt and streptomycin sulfate (Gibco, Grand Island, NY, USA). All other chemicals used were of biochemical reagent grade.

3.2. Plant material

Dracaena thalioides was purchased from Bigup Palm Tree Market (Ibusuki, Kagoshima, Japan) in 2009. A voucher specimen is deposited in our laboratory (voucher No. DT-2009-001, Department of Medicinal Pharmacognosy).

3.3. Extraction and isolation

The underground parts of *D. thalioides* (8.7 kg) were extracted with hot MeOH (36 l x 2). After the solvent was concentrated under reduced pressure, the MeOH extracted (460 g) was passed through a Diaion HP-20 column eluted with H_2O–MeOH (7:3) (5 l), H_2O–MeOH (1:1) (3 l), MeOH (3 l), EtOH (3 l), and EtOAc (3 l),

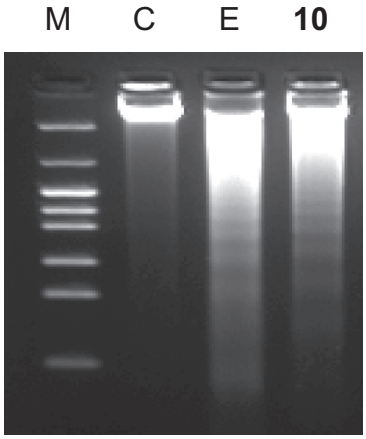

Fig. 3. Induction of DNA fragmentation by **10** in HL-60 cells. HL-60 cells were incubated at 37 °C for 18 h with 10 μM of **10**, or 15 μM of etoposide (E). DNA was then extracted and used for agarose gel electrophoresis. M: DNA marker. C: control.

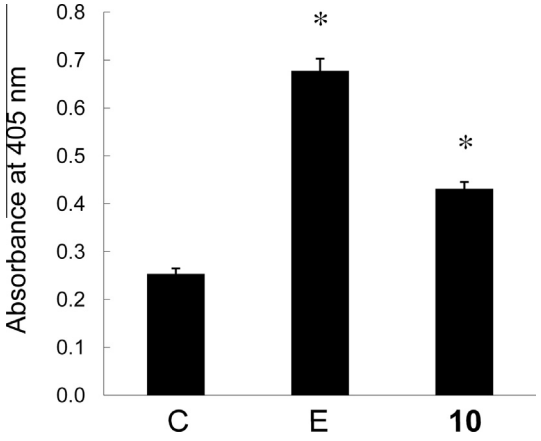

Fig. 4. Caspase-3 activity in the lysates of cells treated with **10** or etoposide. HL-60 cells were incubated at 37 °C for 18 h with 10 μM of **10** or 15 μM of etoposide (E). The data are present as the mean ± SEM of three experiments. Results where $p < 0.05$ were considered significantly different from the control group and are marked with *.

respectively. The MeOH eluate portion (50 g) was subjected to silica gel CC, eluted with $CHCl_3$–MeOH (9:1, 4:1, 2:1, 1:1) and MeOH alone, to give 14 fractions (A–N). Fraction B was applied to an ODS silica gel column eluted with MeOH–H_2O (6:4; 8:2) and MeCN–H_2O (1:3, 1:2) to give **10** (11.7 mg) and **14** (13.9 mg). Fraction C was separated by ODS silica gel CC eluted with MeOH–H_2O (6:4) and by silica gel CC eluted with $CHCl_3$–MeOH–H_2O (90:10:1, 50:10:1) to give **3** (11.7 mg), **15** (16.4 mg), and **16** (11.7 mg). Fraction D was purified by ODS silica gel CC eluted with MeOH–H_2O (6:4; 8:2) to give **2** (70 mg) and **11** (50 mg). Fraction E was sepa-

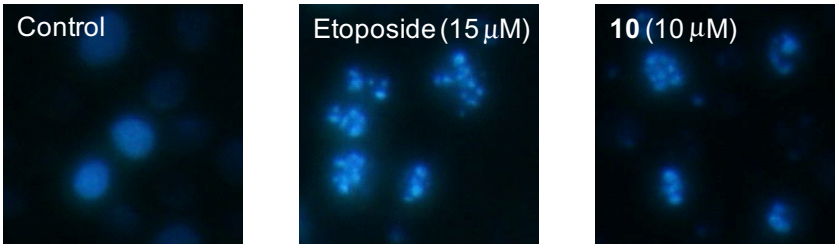

Fig. 2. Morphology of representative fields of HL-60 cells stained with DAPI after treatment with 10 μM of **10**, or 15 μM of etoposide for 48 h to evaluate fragmented and condensed nuclear chromatins.

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rated by ODS silica gel CC eluted with MeOH–H_2O (6:4; 8:2) and MeCN–H_2O (2:5) to afford **6** (5.5 mg) and **7** (3.3 mg). Fraction F was subjected to ODS silica gel CC eluted with MeOH–H_2O (6:4; 8:2) and by silica gel CC eluted with $CHCl_3$–MeOH–H_2O (40:10:1; 30:10:1) to give **1** (17.8 mg), **12** (4.7 mg), and **13** (3.9 mg). Fraction H was applied to an ODS silica gel column eluted with MeOH–H_2O (8:2) to yield **9** (26.2 mg). Fraction I was purified by ODS silica gel CC eluted with MeOH–H_2O (2:3; 8:2) and by silica gel CC eluted with $CHCl_3$–MeOH–H_2O (90:10:1; 40:10:1) to give **4** (198 mg), **5** (12.0 mg), **8** (28.2 mg), and **18** (6.3 mg). Fraction K was separated by silica gel CC eluted with $CHCl_3$–MeOH–H_2O (40:10:1) and by ODS silica gel CC eluted with MeCN–H_2O (1:3) to give **17** (4.0 mg).

3.4. Compound **1**

(25*R*)-17α-hydroxyspitost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-*O*-[α-L-rhamnopyranosyl-(1→3)]-4-*O*-acetyl-β-D-glucopyranoside (**1**); amorphous solid; $[\alpha]_D^{25}$ −81.8 (*c* 0.10, MeOH); IR ν_{max} (film) cm^{-1}: 3416 (OH), 2953 (CH), 1747 (C=O), 1378, 1240, 1039; ^1H NMR (500 MHz, C_5D_5N): δ 5.31 (1H, *br d*, *J* = 4.7 Hz, H-6), 3.86 (1H, *m*, $W_{1/2}$ = 19.3 Hz, H-3), 1.23 (3H, *d*, *J* = 7.2 Hz, Me-21), 1.06 (3H, *s*, Me-19), 0.96 (3H, *s*, Me-18), 0.69 (3H, *d*, *J* = 5.7 Hz, Me-27); For ^1H NMR spectroscopic data of the sugar moiety, see Table 1; for ^{13}C NMR (125 MHz, C_5D_5N) spectroscopic data, see Tables 1 and 2; HRESI-TOFMS *m*/*z* 949.4741 $[M + Na]^+$ (calcd for $C_{47}H_{74}O_{18}Na$, 949.4773).

3.5. Alkaline methanolysis of **1**

Compound **1** (12.9 mg) was treated with 3% NaOMe in MeOH (5 ml) at room temperature for 1 h. The reaction mixture was neutralized by passage through an Amberlite IR-410 (Organo, Tokyo, Japan) column and purified by silica gel CC eluted with $CHCl_3$–MeOH–H_2O (90:30:1) to give **8** (9.9 mg).

3.6. Compound **2**

(25*R*)-17α-hydroxyspitost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-*O*-[α-L-rhamnopyranosyl-(1→3)]-4,6-*O*-diacetyl-β-D-glucopyranoside (**2**); amorphous solid; $[\alpha]_D^{25}$ −85.6 (*c* 0.10, MeOH); IR ν_{max} (film) cm^{-1}: 3389 (OH), 2928 (CH), 1747 (C=O), 1455, 1375, 1240, 1042; ^1H NMR (500 MHz, C_5D_5N): δ 5.33 (1H, *br d*, *J* = 4.8 Hz, H-6), 3.86 (1H, *m*, $W_{1/2}$ = 21.2 Hz, H-3), 1.23 (3H, *d*, *J* = 7.2 Hz, Me-21), 1.07 (3H, *s*, Me-19), 0.96 (3H, *s*, Me-18), 0.69 (3H, *d*, *J* = 5.6 Hz, Me-27); For ^1H NMR spectroscopic data of the sugar moiety, see Table 1; for ^{13}C NMR (125 MHz, C_5D_5N) spectroscopic data, see Tables 1 and 2; HRESI-TOFMS *m*/*z* 991.4838 $[M + Na]^+$ (calcd for $C_{49}H_{76}O_{19}Na$, 991.4879).

3.7. Alkaline methanolysis of **2**

Compound **2** (12.2 mg) was subjected to alkaline methanolysis as described for **1** to give **8** (9.0 mg).

3.8. Compound **3**

(25*R*)-3β-hydroxyspitost-5-en-1β-yl β-D-xylopyranoside (**3**); amorphous solid; $[\alpha]_D^{25}$ −75.6 (*c* 0.10, MeOH); IR ν_{max} (film) cm^{-1}: 3349 (OH), 2928 (CH), 1650, 1454, 1377, 1241, 1040; ^1H NMR (500 MHz, C_5D_5N): δ 5.59 (1H, *br d*, *J* = 5.4 Hz, H-6), 4.54 (1H, *m*, H-16), 3.89 (1H, *m*, $W_{1/2}$ = 22.8 Hz, H-3), 3.86 (1H, *dd*, *J* = 11.6, 3.8 Hz, H-1), 1.30 (3H, *s*, Me-19), 1.13 (3H, *d*, *J* = 6.9 Hz, Me-21), 0.86 (3H, *s*, Me-18), 0.69 (3H, *d*, *J* = 5.2 Hz, Me-27); For ^1H NMR spectroscopic data of the sugar moiety, see Table 1; for ^{13}C NMR (125 MHz, C_5D_5N) spectroscopic data, see Tables 1 and 2; HRESI-TOFMS *m*/*z* 585.3400 $[M + Na]^+$ (calcd. for $C_{32}H_{50}O_8Na$, 585.3403).

3.9. Acid hydrolysis of **3**

A solution of **3** (9.5 mg) in 1 M HCl (dioxane–H_2O, 1:1, 2 ml) was heated at 95 °C for 1 h under an Ar atmosphere. After cooling, the reaction mixture was neutralized by passage through an Amberlite IRA-96SB (Organo) column and applied to a Diaion HP-20 column, eluted with H_2O–MeOH (3:2) followed by EtOH–Me_2CO (1:1), to yield an aglycone fraction and a sugar fraction (4.0 mg). The aglycone fraction was applied to a silica gel column eluted with hexane–Me_2CO (1:1) to give ruscogenin (3.8 mg). The sugar fraction was analyzed by HPLC under the following conditions: column, Capcell Pak NH_2 UG-80 (4.6 mm i.d. ×250 mm, 5 μm, Shiseido, Tokyo, Japan); solvent, MeCN–H_2O (17:3); flow rate, 1.0 ml/min; detection, OR. Identification of D-xylose present in the sugar fraction was carried out by comparing the retention time (t_R) and optical rotation with those of an authentic sample. t_R (min): 9.4 (D-xylose, positive optical rotation).

3.10. Compound **4**

(25*R*)-3β-hydroxyspitost-5-en-1β-yl *O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-β-D-xylopyranoside (**4**); amorphous solid; $[\alpha]_D^{25}$ −82.0 (*c* 0.10, MeOH); IR ν_{max} (film) cm^{-1}: 3243 (OH), 2925 (CH), 1649, 1453, 1375, 1238, 1043; ^1H NMR (500 MHz, C_5D_5N): δ 5.58 (1H, *br d*, *J* = 5.5 Hz, H-6), 4.54 (1H, *m*, H-16), 3.87 (1H, *m*, $W_{1/2}$ = 19.9 Hz, H-3), 3.77 (1H, *dd*, *J* = 11.6, 3.8 Hz, H-1), 1.42 (3H, *s*, Me-19), 1.15 (3H, *d*, *J* = 6.9 Hz, Me-21), 0.92 (3H, *s*, Me-18), 0.68 (3H, *d*, *J* = 5.2 Hz, Me-27); For ^1H NMR spectroscopic data of the sugar moiety, see Table 1; for ^{13}C NMR (125 MHz, C_5D_5N) spectroscopic data, see Tables 1 and 2; HRESI-TOFMS *m*/*z* 863.4412 $[M + Na]^+$ (calcd for $C_{43}H_{68}O_{16}Na$, 863.4405).

3.11. Compound **5**

(25*R*)-3β-hydroxyspitost-5-en-1β-yl *O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (**5**); amorphous solid; $[\alpha]_D^{25}$ −73.1 (*c* 0.10, MeOH); IR ν_{max} (film) cm^{-1}: 3332 (OH), 2925 (CH), 1649, 1454, 1375, 1260, 1073; ^1H NMR (500 MHz, C_5D_5N) : δ 5.55 (1H, *br d*, *J* = 5.3 Hz, H-6), 4.46 (1H, *m*, H-16), 3.89 (1H, *dd*, *J* = 13.3, 3.9 Hz, H-1), 3.78 (1H, *m*, $W_{1/2}$ = 19.6 Hz, H-3), 1.45 (3H, *s*, Me-19), 1.10 (3H, *d*, *J* = 6.9 Hz, Me-21), 0.92 (3H, *s*, Me-18), 0.69 (3H, *d*, *J* = 5.4 Hz, Me-27); For ^1H NMR spectroscopic data of the sugar moiety, see Table 1; for ^{13}C NMR (125 MHz, C_5D_5N) spectroscopic data, see Tables 1 and 2; HRESI-TOFMS *m*/*z* 761.4116 $[M + Na]^+$ (calcd for $C_{39}H_{62}O_{13}Na$, 761.4088).

3.12. Acid hydrolysis of **4** and **5**

Compound **4** (9.2 mg) and **5** (5.6 mg) were independently subjected to acid hydrolysis using the method described for **3** to give an aglycone (ruscogenin: 2.8 mg from **4** and 1.5 mg from **5**) and sugar fractions (4.0 mg from **4** and 3.0 mg from **5**). HPLC analysis of the sugar fractions under the same conditions used for **3** showed the presence of L-rhamnose and D-xylose in that of **4**, and of L-rhamnose and D-glucose in that of **5**. t_R (min): 7.6 (L-rhamnose, negative optical rotation), 9.4 (D-xylose, positive optical rotation), and 14.6 (D-glucose, positive optical rotation).

3.13. Compound **6**

(23*S*,24*S*)-3β,23-dihydroxy-1β-[*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1→2)-*O*-[3-*O*-acetyl-β-D-xylopyranosyl-(1→3)]-α-L-

Table 1
^{1}H and ^{13}C NMR chemical shift assignments for the sugar moiety of **1–6** in C_5D_5N.[a]

1				**2**				**3**			
		^{1}H	^{13}C			^{1}H	^{13}C			^{1}H	^{13}C
Glc	1	4.90, d (7.7)	99.8	Glc	1	4.89, d (7.9)	100.0	Xyl	1	4.85, d (7.6)	102.4
	2	4.05, dd (8.8, 7.7)	78.3		2	4.07, dd (8.6, 7.9)	78.1		2	4.00, dd (8.8, 7.6)	75.2
	3	4.29, m	82.2		3	4.28, dd (9.6, 8.6)	81.8		3	4.15, dd (8.8, 8.8)	78.7
	4	5.41, dd (9.6, 9.6)	71.3		4	5.38, dd (9.6, 9.6)	70.5		4	4.26, m	71.2
	5	3.86, m	75.8		5	3.91, m	72.3		5a	4.39, dd (11.2, 5.4)	67.5
	6a	4.09, dd (12.2, 2.6)	62.1		6a	4.56, dd (12.2, 5.1)	63.1		b	3.68, dd (11.2, 10.6)	
	b	4.02, dd (12.2, 5.8)			b	4.35, dd (12.2, 2.4)					
Rha (I)	1	5.79, br s	102.6	Rha (I)	1	5.79, br s	102.7				
	2	4.73, br d (2.9)	72.3		2	4.73, br d (2.0)	72.3				
	3	4.47, dd (9.3, 2.9)	72.7		3	4.50, m	72.7				
	4	4.31, dd (9.3, 9.3)	73.6		4	4.31, m	73.7				
	5	4.82, dq (9.3,6.2)	70.0		5	4.79, m	70.1				
	6	1.73, d (6.2)	18.5		6	1.73, d (6.2)	18.6				
Rha (II)	1	5.66, br s	103.5	Rha (II)	1	5.65, br s	103.5				
	2	4.68, br d (3.0)	72.5		2	4.68, br d (2.9)	72.5				
	3	4.45, m	72.3		3	4.43, dd (9.0, 2.9)	72.3				
	4	4.25, m	73.7		4	4.23, dd (9.0, 9.0)	73.6				
	5	4.24, m	70.7		5	4.22, dq (9.0, 5.6)	70.7				
	6	1.64, d (5.2)	18.4		6	1.63, d (5.6)	18.4				
	Ac	2.18, s	21.4		Ac	2.19, s	21.3				
			170.4				170.2				
						2.05, s	20.7				
							170.6				
4				**5**				**6**			
Xyl (I)	1	4.70, d (7.6)	100.6	Glc	1	4.89, d (7.5)	101.5	Ara	1	4.63, d (7.7)	100.0
	2	4.14, m	75.9		2	4.28, dd (8.8, 7.5)	76.7		2	4.51, dd (7.7, 9.1)	73.0
	3	3.99, m	88.5		3	4.22, dd (9.4, 8.8)	79.8		3	4.01, m	84.7
	4	3.99, m	69.5		4	4.01, dd (9.4, 9.4)	72.7		4	4.35, m	69.8
	5a	4.32, m	66.5		5	3.86, m	77.8		5a	4.22, m	67.4
	b	4.52, m			6a	4.53, dd (11.2, 2.7)	63.6		b	3.66, m	
					b	4.29, dd (11.2, 4.2)					
Rha	1	6.44, br s	101.7	Rha	1	6.50, br s	99.9	Rha	1	6.40, br s	97.6
	2	4.78, br d (3.3)	72.4		2	4.74, br d (3.2)	72.6		2	6.03, dd (3.5, 1.6)	70.1
	3	4.58, dd (9.3, 3.3)	72.4		3	4.64, dd (9.3, 3.2)	72.6		3	5.90, dd (10.2, 3.5)	70.3
	4	4.31, dd (9.3, 9.3)	74.1		4	4.35, dd (9.3, 9.3)	74.2		4	5.63, dd (10.2, 10.2)	71.8
	5	4.81, dd (9.3, 6.1)	69.6		5	4.92, dq (9.3, 6.2)	69.4		5	4.94, m	66.5
	6	1.77, d (6.1)	19.3		6	1.79, d (6.2)	19.1		6	1.42, d (6.8)	18.1
Xyl (II)	1	4.94, d (7.6)	105.2					Xyl	1	4.83, d (7.8)	105.8
	2	3.99, m	74.7						2	3.81, dd (9.2, 7.8)	72.3
	3	4.08, dd (8.7, 8.7)	78.4						3	5.58, dd (9.2, 9.2)	79.1
	4	4.13, m	70.6						4	4.07, m	69.1
	5a	4.25, dd (10.8, 5.1)	67.2						5a	4.24, m	66.7
	b	3.68, dd (10.8, 10.8)							b	3.63, m	
								Glc	1	5.38, d (7.9)	106.1
									2	4.09, m	75.8
									3	4.23, m	78.5
									4	4.19, m	71.5
									5	3.88, m	78.6
									6a	4.46, dd (11.5, 2.1)	62.6
									b	4.32, dd (11.5, 2.1)	
									Ac	2.12, s	20.7
											170.3
										2.04, s	20.8
											170.5
										1.99, s	20.6
											170.4
										1.92, s	21.0
											170.4

[a] Values in parentheses are coupling constants in Hz.

arabinopyranosyl)oxy]spirosta-5,25(27)-dien-24-yl β-D-glucopyranoside (**6**); amorphous solid; $[\alpha]_D^{25}$ -50.7 (*c* 0.10, MeOH); IR ν_{max} (film) cm^{-1}: 3251 (OH), 2924 (CH), 1747 (C=O), 1600, 1514, 1371, 1259, 1046; ^{1}H NMR (500 MHz, C_5D_5N) : δ 5.63 (1H, *br d*, *J* = 4.4 Hz, H-6), 5.22 (1H, *br s*, H-27a), 5.03 (1H, *br s*, H-27b), 4.86 (1H, *d*, *J* = 11.5 Hz, H-26a), 4.00 (1H, *d*, *J* = 11.5 Hz, H-26b), 4.63 (1H, *m*, H-16), 4.82 (1H, *br s*, H-24), 3,99 (1H, *br s*, H-23), 3.89 (1H, *overlapping*, H-3), 3.81 (1H, *dd*, *J* = 11.9, 3.8 Hz, H-1), 1.35 (3H, *s*, Me-19), 1.08 (3H, *d*, *J* = 7.0 Hz, Me-21), 0.99 (3H, *s*, Me-18); For ^{1}H NMR spectroscopic data of the sugar moiety, see Table 1; for ^{13}C NMR

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Table 2
^{13}C NMR chemical shift assignments for the aglycone moiety of **1**–**6** in C_5D_5N.

Position	1	2	3	4	5	6
1	37.4	37.5	83.2	84.0	83.7	83.7
2	29.9	30.0	37.6	37.4	37.7	37.6
3	78.1	78.6	68.0	68.2	68.0	67.9
4	38.6	38.7	43.8	43.7	43.8	43.8
5	140.7	140.7	139.4	139.3	139.4	139.3
6	121.8	121.9	125.4	124.7	124.7	124.9
7	32.4	32.4	31.9	31.8	31.8	31.9
8	32.3	32.3	33.0	33.1	33.1	32.9
9	50.2	50.2	50.3	50.3	50.4	50.2
10	37.1	37.1	42.8	42.7	42.8	42.8
11	20.9	20.9	23.9	24.2	24.1	23.9
12	32.1	32.1	40.5	40.4	40.5	40.4
13	45.1	45.1	40.2	40.3	40.2	40.7
14	53.0	53.0	56.8	56.9	57.0	56.6
15	31.8	31.8	32.4	32.3	32.3	32.4
16	90.0	90.0	81.1	81.1	81.1	82.9
17	90.1	90.1	63.1	63.1	63.0	61.5
18	17.1	17.1	16.7	16.8	16.8	16.8
19	19.4	19.4	15.0	15.0	15.0	14.8
20	44.7	44.8	41.9	42.0	42.0	37.4
21	9.7	9.7	14.7	15.0	14.9	14.7
22	109.8	109.8	109.2	109.2	109.2	111.8
23	32.1	32.1	31.8	31.8	31.9	70.2
24	28.8	28.8	29.6	30.6	29.2	82.1
25	30.4	30.4	30.6	29.2	30.6	143.6
26	66.7	66.7	66.8	66.8	66.8	61.5
27	17.3	17.3	17.3	17.3	17.3	114.1

Table 3
Cytotoxic activities of the isolated compounds **1**–**18**, cisplatin, and etoposide against HL-60 cells.

Compounds	IC_{50} (μM)*
1	7.64 ± 0.59
2	>20
3	7.38 ± 0.78
4	11.3 ± 1.21
5	17.3 ± 2.99
6	1.66 ± 0.20
7	>20
8	6.36 ± 0.14
9	12.3 ± 2.56
10	0.38 ± 0.04
11	0.47 ± 0.04
12	6.00 ± 1.22
13	2.73 ± 0.42
14	4.45 ± 0.39
15	0.74 ± 0.05
16	9.34 ± 2.93
17	7.85 ± 0.43
18	9.45 ± 2.22
Cisplatin	1.40 ± 0.08
Etoposide	0.38 ± 0.06

* Data are present as the mean ± SEM of three experiments performed in triplicate.

(125 MHz, C_5D_5N) spectroscopic data, see Tables 1 and 2; HRESI-TOFMS m/z 1223.5079 $[M + Na]^+$ (calcd for $C_{57}H_{84}O_{27}Na$, 1223.5098).

3.14. HL-60 cell culture assay

HL-60 cells were maintained in RPMI 1640 medium containing heat-inactivated 10% (v/v) fetal bovine serum (FBS) supplemented with 100 units/ml penicillin G sodium salt and 100 μg/ml streptomycin sulfate. For the cytotoxicity assay, the cells were washed and re-suspended in the medium at a density of 4×10^4 cells/ml, and 196 μl of the cell suspension was placed in each well of a 96-well flat-bottom plate. The cells were incubated in a humidified air/CO_2 (19:1) atmosphere for 24 h at 37 °C. After incubation, 4 μl of an EtOH–H_2O (1:1) solution containing the test samples was added to give final concentrations of 0.1–20 μM and 4 μl of EtOH–H_2O was added into control wells. The cell growth was evaluated by the modified MTT assay procedure established by Sargent and Taylor (Sargent and Taylor, 1989). A dose–response curve was plotted for **1**, **3**–**6**, and **8**–**18**, which showed less than 50% cell growth at a sample concentration of 20 μM, and the concentration at which 50% inhibition (IC_{50}) was observed was calculated. Each assay was done in triplicate.

3.15. Assay for detection of DNA fragmentation

The cells were incubated at 37 °C for 18 h with **10** (10 μM) and etoposide (15 μM). DNA was extracted with a commercially available kit (Wizard Genomic DNA Purification Kit, Promega, WI, USA). In brief, cells (2×10^6 cells) were centrifuged for 5 min at 10,000*g*. The cell pellet was re-suspended in nuclei lysis solution (600 μl) Then, 4 mg/ml RNaseA solution (3 μl) was added to the cell lysate, and the solution was incubated at 37 °C for 15 min. Protein precipitation solution (200 μl) was added to the RNaseA-treated cell lysate, and the mixture was incubated for 5 min on ice and centrifuged at 10,000*g* for 5 min. The supernatant was transferred to a clean 1.5 ml microcentrifuge tube containing *i*PrOH (600 μl) and was mixed by inversion. After centrifugation at 10,000*g* for 5 min, DNA was visible as a small white pellet and it was washed with EtOH–H_2O (70:30, v/v). Finally, the pellet was re-suspended in DNA rehydration solution (25 μl), incubated at 65 °C for 1 h, and stored at −20 °C until used. The sample (15 μl) was used for 2% agarose gel electrophoresis in 40 mM Tris–acetate buffer (pH 7.4) at 50 V for 1 h. A DNA molecular weight marker (pH marker, Takara, Shiga, Japan) and DNA from apoptotic HL-60 cells induced by etoposide (10 μM) were used for calibration. The DNA fragmentation pattern was examined in photographs taken under UV illumination.

3.16. Assay for caspase-3 activation

The activity of caspase-3 was measured by using a commercially available kit (Appocyto Caspase-3 Colorimetric Assay Kit, MBL, Aichi, Japan). HL-60 cells (2×10^6) were treated with **10** (10 μM) and etoposide (15 μM) for 6 h, and the cells were centrifuged and collected. Cell pellets were suspended in ice-cold cell lysis buffer (60 μl), and incubated on ice for 10 min. This cell pellet suspension was centrifuged at 10,000*g* for 5 min and the supernatant was collected. The cell lysate (50 μl, equivalent to 200 μg protein) was mixed with reaction buffer (2×50 μl) containing the substrates for caspase-3 [DEVD-*p*NA (*p*-nitroanilide)]. After incubation for 2 h at 37 °C, the absorbance at 405 nm of the liberated chromophore *p*NA was measured using a microplate reader. The activity of caspase-3 was evaluated in triplicate.

3.17. Statistical analysis

For statistical analysis, one-way analysis of variance (ANOVA) followed by Dunnett's test was performed. A probability (p) value of less than 0.05 was considered to represent a statistically significant difference.

References

Agrawal, P.K., Jain, D.C., Gupta, R.K., Thakur, R.S., 1985. Carbon-13 NMR spectroscopy of steroidal sapogenins and steroidal saponins. Phytochemistry 24, 2479–2496.

Asano, T., Murayama, T., Hirai, Y., Shoji, J., 1993. Comparative studies on the constituents Ophiopogonis tuber and its congeners. VIII. Studies on the glycosides of the subterranean part of *Ophiopogon japonicus* Ker-Gawler cv. Nanus. (2). Chem. Pharm. Bull. 41, 566–570.

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Liu, H., Chou, G.X., Wu, T., Guo, Y.L., Wang, S.C., Wang, C.H., Wang, Z.T., 2009. Steroidal sapogenins and glycosides from the rhizomes of *Dioscorea bulbifera*. J. Nat. Prod. 72, 1964–1968.

Mimaki, Y., Inoue, T., Kuroda, M., Sashida, Y., 1996a. Steroidal saponins from *Sansevieria trifasciata*. Phytochemistry 43, 1325–1331.

Mimaki, Y., Takaashi, Y., Koroda, M., Sashida, Y., Nikaido, T., 1996b. Steroidal saponins from *Nolina recurvata* stems and their inhibitory activity on cyclic AMP phosphodiesterase. Phytochemistry 42, 1609–1615.

Mimaki, Y., Kuroda, M., Ide, A., Kameyama, A., Yokosuka, A., Sashida, Y., 1999. Steroidal saponins from the aerial parts of *Dracaena draco* and their cytostatic activity on HL-60 cells. Phytochemistry 50, 805–813.

Mimaki, Y., Yokosuka, A., Kuroda, M., Sashida, Y., 2001. Cytotoxic activities and structure–cytotoxic relationships of steroidal saponins. Biol. Pharm. Bull. 24, 1286–1289.

Monti, D., Pisvejcova, A., Kren, V., Lama, M., Riva, S., 2004. Generation of an α-L-rhamnosidase library and its application for the selective derhamnosylation of natural products. Biotechnol. Bioeng. 87, 763–771.

Sargent, J.M., Taylor, C.G., 1989. Appraisal of the MTT assay as a rapid test of chemosensitivity in acute myeloid leukaemia. J. Cancer 60, 206–210.

Tapondjou, L.A., Ponou, K.B., Teponno, R.B., Mbiantcha, M., Djoukeng, J.D., Nguelefack, T.B., Watcho, P., Cadenas, A.G., Park, H., 2008. In vivo anti-inflammatory effect of a new steroidal saponin, mannioside A, and its derivatives isolated from *Dracaena mannii*. J. Arch. Pharm. Res. 31, 653–658.

Tran, Q.L., Tezuka, Y., Banskota, A.H., Tran, Q.K., Saiki, I., Kadota, S., 2001. New spirostanol steroids and steroidal saponins from roots and rhizomes of *Dracaena angustifolia* and their antiproliferative activity. J. Nat. Prod. 64, 1127–1132.

Tsukamoto, Y. (ed.), 1988. The Grand Dictionary of Horticulture, vol. 3. Shogakukan, Tokyo, pp. 401–405.

Wang, K.W., Zhang, H., Shen, L.Q., Wang, W., 2011. Novel steroidal saponins from *Liriope graminifolia* (Linn.) Baker with anti-tumor activities. Carbohydr. Res. 346, 253–258.

Yokosuka, A., Mimaki, Y., Sashida, Y., 2000. Steroidal saponins from *Dracaena surculosa*. J. Nat. Prod. 63, 1239–1243.

Yokosuka, A., Mimaki, Y., 2009. Steroidal saponins from the whole plants of *Agave utahensis* and their cytotoxic activity. Phytochemistry 70, 807–815.

Yokosuka, A., Sekiguchi, A., Mimaki, Y., 2013. Chemical constituents of the leaves of *Dracaena thalioides*. Nat. Prod. Commun. 8, 315–318.

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